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Motor unit loss estimation by the multipoint incremental MUNE method in children with spinal muscular atrophy – A preliminary study

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Abstract

Quantitative EMG reflects denervation of muscles after lower motor neuron degeneration in spinal muscular atrophy (SMA) but does not reflect actual motor unit loss. The aim of our study was to assess the value of the multipoint incremental motor unit number estimation (MUNE) method in the modification by Shefner in estimating motor unit loss in SMA. The number of motor units, the mean amplitude of an average surface-detected single motor unit potential (SMUP), and the amplitude of compound motor action potentials (CMAP) were estimated in 14 children with SMA in the abductor pollicis brevis (ABP). Significant differences in MUNE values and SMUP and CMAP amplitude were found between the SMA and control groups ($P < 0.0001$). MUNE values correlated with Hammersmith Functional Motor Scale (HFMS) scores ($P < 0.05$). Increased SMUP amplitude values correlated with decreased HFMS scores ($P < 0.05$). The study confirms that MUNE method in the modification by Shefner is a useful tool reflecting motor unit loss in SMA, and it is easy to perform and well tolerated. MUNE and SMUP amplitude seemed to be sensitive parameters reflecting motor dysfunction in SMA but a longitudinal study in a larger number of subjects is needed.

Keywords: Spinal muscular atrophy; Motor unit number estimation; MUNE; Motor unit

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease caused by a mutation in and a functional loss of the *survival motor neuron 1* (*SMN1*) gene and retention of twin *SMN2* gene [1]. A homozygous deletion/conversion involving exon 7 in *SMN1* has been demonstrated in more than 96% of patients with SMA. *SMN2* differs from *SMN1* by a single nucleotide in exon 7 that alters the splicing pattern [2]. The protein product lacking exon 7 is not functional and is rapidly degraded, and thus the *SMN2* gene produces less essential cellular protein than *SMN1* gene [3]. The *SMN2* gene can exist in a number of copies (1–6 in SMA patients), partially compensating for the loss of the *SMN1* gene [4,5]. As a result, SMA is the effect of a low and variable amount of the SMN protein produced by the *SMN2* gene [6].

A number of mouse models have been created to evaluate the pathogenesis of SMA by expressing a low level of human SMN protein from a transgenic locus. Analysis of these models provided clear support for the notion that motor neurons are particularly vulnerable to low SMN protein levels [6–9]. Selective degeneration of lower motor neurons in the ventral horn of the spinal cord is the key pathological abnormality in SMA. The loss of alpha motor neurons is the main reason of progressive muscle denervation, resulting in muscle atrophy, limb paresis and finally paralysis. Electrodiagnostic studies are routinely used for the differential diagnosis of SMA and other forms of motor neuron disease by reflecting primary pathologic (denervation) and compensatory reinnervation changes that take place after lower motor neuron loss. Typical electromyography (EMG) studies in patients with SMA show fibrillations and fasciculations at rest and an increased mean duration and amplitude of motor units during slight effort, although EMG findings may be different in different forms of SMA. In Werdnig–Hoffmann disease (SMA1) and the intermediate form (SMA2), spontaneous rhythmic firing of motor units was recorded, and complex repetitive discharges

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occurred in long-standing cases (SMA3) [10]. Parameters of individual motor unit potentials (MUPs) also vary between different forms of the disease. In SMA1, EMG shows some short and low amplitude potentials in addition to long duration, high amplitude potentials. In the SMA3 cases, MUP parameters are shifted to longer durations and higher amplitudes [10]. It has been well established that spontaneous activity manifesting as fibrillation or positive sharp waves is due to active denervation, and increased MUP amplitude, area and duration are caused by a compensatory process of reinnervation, although values of these parameters depend on the disease stage. This reorganization of the motor unit as a result of coexisting processes of denervation and reinnervation initially allows full compensation but finally leads to decompensation of innervation. In the early stages of disease the MUAPs parameters are increased because of efficient reinnervation of muscle fibers by collateral axonal sprouting (for amplitude up to 1000% normal value, for duration up to 150% normal value) [11]. In advanced stages of SMA, motor unit action potential area and amplitude may approach normal ranges because of decompensation of innervation and disintegration of the motor unit. However, quantitative EMG findings reflect denervation and reinnervation changes that take place after lower motor neuron degeneration and do not reflect actual motor unit loss. Methods of motor unit number estimation (MUNE) have long been of interest as a measure of lower motor neuron loss. Assessing the change in the number of motor units is particularly valuable for the evaluation of motor neuron disease severity and progression. Various techniques for MUNE have been developed but they have been very rarely used in SMA, although some of the methods are noninvasive and well tolerated in contrary to routine needle EMG which is painful, particularly in children. Among different methods such as manual incremental stimulation [12] with MUNE obtained by averaging several potentials of an increased amplitude recorded within one site, spike-triggering averaging method based on needle EMG, and multiple point stimulation with motor nerve stimulation at multiple sites along the nerve, we chose the incremental multipoint MUNE method (in the modification by Shefner) [13] that seems non-invasive, well tolerated (stimuli of a very low intensity are usually used) and reproducible [14]. Our experience regarding reproducibility of the multipoint incremental MUNE method was described in our previous paper. In 20 subjects, intrarater MUNE retest was performed 1–4 weeks after the initial examination. The mean difference between the first and the second MUNE result was 7% with a 95% confidence interval of 4%–16% [14].

The aim of our study was to assess estimate the value of multipoint incremental MUNE method used to determine motor unit loss in SMA and to estimate the values of MUNE, the mean amplitude of an average surface-detected single motor unit potential (SMUP), and the amplitude of compound motor action potentials (CMAP) in SMA in comparison to the normal range. Another objective was to determine whether those electrophysiological parameters reflect the loss of motor function estimated based on the functional motor scale and the age at the time of immobilization. We also analyzed relations between the patient age/age at the time of first symptoms and MUNE, SMUP and CMAP.

2. Patients and methods

The study group consisted of 14 right-handed non-ambulant patients with genetically confirmed SMA (10 with SMA3, 4 with SMA2) at the mean age of 12.4 ± 4.6 years (range 6–18 years), including 5 males (35.7%). The mean patient age at the disease onset was 15.6 months (10–24 months), and the mean duration of the disease was 11.1 years (5–16 years). The mean age at loss of independent ambulation in patients with SMA3 was 9.5 years (6–14 years). Motor functions were evaluated using the Hammersmith Functional Motor Scale (HFMS) for SMA [15]. Activities included in the scale were rolling, sitting, lifting head from prone and supine, getting up from lying, propping on arms, four-point kneeling, crawling, standing, and stepping. Each item scores 0–2 with 0 meaning unable, 1 meaning some adaptation and 2 meaning fully able, with the maximal total score of 40 [16].

The control group consisted of 15 healthy volunteers at the mean age of 25.47 ± 3.4 years (range 18–30 years). The age of patients and controls differed significantly ($P = 0.0003$) but it has been established that the number of motor units remains stable from the neonatal period to the adulthood. Data for normal young subjects are very limited, but the number of motor units is similar to adult values [17] and consistent with anatomic estimates [18]. In case of human motoneurons, cell loss is very slow before 60 years and increases linearly thereafter [19].

All patients were enrolled to the TROPHOS study, and MUNE estimation was performed during Vo visit before the investigational product was administered. The authors have obtained the required permission from the sponsor of the trial to use selected MUNE data. We decided to present an analysis of data that are a subset of data collected during the TROPHOS trial conducted in many centers across the Europe because of the possibility to compare the results with normal ranges established in our EMG Laboratory. Dispersion of MUNE results between different centers has been observed so it is reasonable to create individual reference ranges of MUNE values in every EMG Laboratory. All parents of the patients gave their written informed consent for child participation in MUNE examinations, and the protocol was approved by the Ethics Committee at the Medical University of Warsaw (No KB/18/R/2011).

MUNE tests were performed using the Keypoint Classic Medtronic Functional Diagnostics EMG system. The incremental multipoint MUNE method in the modification by Shefner was chosen for this study because of its non-invasiveness, good tolerance (usually, stimuli of a very low intensity are used) and good reproducibility [14]. Motor fibers of the median nerve of the right (7) or left (7) hand were studied. Disposable, self-adhesive recording electrodes (strips 12 × 22 mm) were placed on the abductor pollicis brevis (APB) muscle innervated by the median nerve (recording area 4 × 7 mm, specific part number 9013L0202, Medtronic). The ground